# A SYSTEMATIC STUDY OF THE *MIMULUS WIENSII* COMPLEX (SCROPHULARIACEAE: *MIMULUS* SECTION *SIMIOLUS*), INCLUDING *M. YECORENSIS* AND *M. MINUTIFLORUS,* NEW SPECIES FROM WESTERN MEXICO

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ABSTRACT

I grew cultures of 14 diverse populations representative of the *Mimulus wiensii* complex of the Sierra Madre of western Mexico and compared them morphologically, cytologically, and as to their ability to hybridize one with another and with a set of six reference populations to which they might be related. The morphological comparisons indicated that *M. wiensii* Vickery was more polymorphic and widespread than previously thought. In addition, the comparisons revealed two morphologically clearly distinct, new species. The cytological studies showed *M. wiensii* to be diploid with $n = 16$ chromosomes, and the new species to be polyploid with $n = 32$ chromosomes for the first species and $n = 32$, $32\pm$, $48\pm$, and $64\pm$ chromosomes for the second. The experimental hybridizations demonstrated that the various populations of *M. wiensii* cross readily with each other but do not cross with the other reference populations or with either of the new species. The two new species do not cross with each other or with any of the reference populations. The first new species is named **M. yecorensis** sp. nov. for its region of occurrence and the second, **M. minutiflorus** sp. nov. for its most prominent characteristic.

RESUMEN

Poblaciones del complejo *Mimulus wiensii* fueron comparadas con respecto a sus características morfológicas, citológicas, y a sus capacidades de formar hibridos. Las poblaciones de la especie *M. wiensii* se encontraron tener $n = 16$ cromosomas y formar hibridos fértiles entre ellos pero non con las otras poblaciones referencia o con *M. yecorensis* y *M. minutiflorus.* Las poblaciones de *M. wiensii* son fenotipicamente más variables y tienen un rango más extenso que originalmente supuesto. **Mimulus yecorensis** sp. nov. se encontró entapetes densos y bajos. Ella posee $n = 32$ cromosomas y casi nunca formó hibridos con otras poblaciones del complejo. **Mimulus minutiflorus** sp. nov. tiene tallos erguidos, delicados, y tiesos con flores muy pequeñas. Ella posee $n = 32$, $32\pm$, $48\pm$, $64\pm$ cromosomas y formó solo un hibrido quando cruzado con otras poblaciones del complejo.

The opening of the Durango-Mazatlan portion of México 40 highway nearly 40 years ago greatly facilitated the botanical exploration of the central Sierra Madre Occidental. The first new *Mimulus* species to come to light was *M. wiensii* Vickery, collected by Delbert Wiens in 1959 (Vickery 1973). Since then, additional collections of *M. wiensii* and distinctive forms apparently related to it have been collected in the Sierra Madre along Route 40 as well as to the north and south of it. The most distinctive populations do not appear to

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match any described species (Grant 1924; Gentry 1947; Kearney and Peebles 1951; Munz 1959; Shreve and Wiggins 1964; Wiggins 1980; Thompson 1993) or any specimens in the herbaria of the University of California-Berkeley (UC, JEPS), Stanford (DS), Harvard (GH), the U.S. National (US), University of Michigan (MICH), and Tucuman (LIL).

In order to elucidate their specific status, these distinctive new populations were compared morphologically, cytologically, and as to their ability to cross with each other, with other members of the *M. wiensii* complex, and with other known *Mimulus* species of the area.

## Materials and Methods

A living collection of 14 cultures of populations of the *M. wiensii* complex plus 6 reference cultures of populations of species to which they might be related was assembled for study in a greenhouse at University of Utah. The populations of the complex came from as far south as Hidalgo and as far north as Arizona, but they came mainly from the central Sierra Madre of western México (Table 1). The study populations provide a good sample of the *M. wiensii* complex with one possible exception, *M. pennellii* H. S. Gentry. My examination of the type specimen of *M. pennellii* suggests to me on morphological grounds that that species is part of the complex. However, unfortunately, it was not obtainable for this experimental study because it grows in the mountains of Sinaloa, which are dangerous due to drug traffic.

The reference cultures included two of *M. wiensii* Vickery, the most abundant species of the complex, two of *M. dentilobus* Rob & Fern, belonging to a related complex occurring to the north, and two of *M. glabratus*—one of diploid *M. glabratus* var. *fremontii* (Benth.) Grant and one of tetraploid *M. glabratus* H.B.K. var. *glabratus*—of the related *M. glabratus* complex which is widespread throughout western Mexico. *Mimulus glabratus* var. *fremontii* includes *M. madrensis* Seem, (based on my study of the type of *M. madrensis*), thus disposing of the only other published species that was potentially part of the *M. wiensii* complex.

Seeds of the study populations were collected by me and my collaborators over a period of years (Table 1). The seeds were sown and the plants propagated in a greenhouse at University of Utah.

The morphological characteristics of the plants of the study populations and of the reference populations were observed and compared as the plants developed, flowered, and set seed.

Chromosome counts for the 14 populations of the *M. wiensii* complex used in this study were made from various stages of microsporogenesis using standard aceto-carmine squash methods as previ-

TABLE 1. ORIGINS AND CHROMOSOME COUNTS OF THE STUDY POPULATIONS OF THE *MIMULUS WIENSII* COMPLEX (*MIMULUS*, SECTION *SIOLUS*). All populations were grown under my culture numbers. Vouchers are in the Garrett Herbarium of the University of Utah (UT). An asterisk indicates populations used in the experimental hybridizations.

New Collections of the *M. wiensii* complex:

*Mimulus wiensii* Vickery. *n* = 16—MÉXICO: Hidalgo, El Chico National Park north of Pachuca, culture *13094** (*P. Bretting, s.n.*, 11 Oct. 1979); Chihuahua, Barranca del Cobre, culture *13095** (*R. A. Bye 9725*, 31 May 1980); Créel, culture *13008** (*R. A. Bye and W. A. Weber 8391*, 19 Oct. 1977); La Cascada near Créel, culture *12197** (*R. K. Vickery, Jr. 2880,* 3 May 1976); Durango, 11 km nw of Santiago Papasquiero, culture *13460* (*R. Díaz 72 and R. D. Worthington 9614*, 9 Jan. 1983); route 40 w of La Ciudad, culture *13485* (*S. Sutherland, s.n.*, 11 June 1984); Lecheria, culture *12206** (*R. K. Vickery, Jr. 2889,* 9 May 1976); km 177 on route 40, culture *12220** (*R. K. Vickery, Jr. 2903,* 12 May 1976); crest of Sierra Madre, route 40, culture *12222** (*R. K. Vickery, Jr. 2905,* 12 May 1976).

*Mimulus dentilobus* Rob. & Fernald. *n* = 16—USA.: AZ, Greenlee, CO, Eagle Creek, culture *13004* (*W. L. Minckley & Assoc. 29-VI-77*).

*Mimulus yecorensis* n. sp. *n* = 32—MÉXICO: Sonora, Yecora, culture *13257** (*D. A. Polhemus, s.n.*, April 24, 1982);

*Mimulus minutiflorus* n. sp. *n* = 32—MÉXICO: Durango, crest of the Sierra Madre, culture *12218** (*R. K. Vickery, Jr. 2901,* 12 May 1976); Morelos, Tepozteco Temple, culture *13518* (*S. Sutherland s.n.*, 8 April 1985); *n* = 32 ±, 48±, 64±—MÉXICO, Durango, El Palmito, culture *7169** (collected with *Breedlove 7231,* March 1965, which is *M. glabratus* H.B.K. var. *glabratus*).

Reference Populations used in this study.

*Mimulus dentilobus* Rob & Fernald. *n* = 16 (Mukherjee et al. 1957, Vickery et al. 1978)— MÉXICO: Sierra Charro, Chihuahua, *5324** (*H. S. Gentry 8073,* April 1948);—USA: AZ, Eagle Creek *13007** (*L. A. McGill 1415,* 7 June 1977).

*Mimulus wiensii* Vickery. *n* = 16 (Mia et al. 1964)—MÉXICO: Durango, crest of Sierra Madre, Highway 40, culture *6272** (Note culture number *6272* = culture *6212*). West of the crest of the Sierra Madre, Highway 40, ca. 1 km west of the previous population, culture number *6273** (*R. K. Vickery, Jr. 2616,* 16 June 1960).

*Mimulus glabratus* var. *fremontii* (Benth.) Grant. *n* = 15 (Vickery et al. 1985)—MÉXICO: Durango, Durango, culture *12215** (*R. K. Vickery, Jr. 2898,* 11 May 1976).

*Mimulus glabratus* H.B.K. var. *glabratus. n* = 31 (Mia et al. 1964)—MÉXICO, Durango, El Salto, culture *6209** (*D. Wiens 2635,* 23 August 1959).

ously described (Vickery et al. 1985). Twenty or more cells were studied from 1–4 plants of each population except for cytogenetically difficult population *7169,* for which 134 cells were studied from 10 plants (Table 2). For each population representative cells were recorded with sketches or camera lucida drawings. The chromosome counts for the 6 reference populations had already been ascertained (Table 1).

For the experimental hybridization study, 16 populations—10 of the study populations and all 6 of the reference populations—were crossed in as many combinations as possible (Table 3). For each cross the pistils of 10 or more flowers of the female parent were

TABLE 2. CHROMOSOME NUMBERS AND THEIR FREQUENCIES FOUND FOR CULTURE *7169* OF THE POPULATION OF *MIMULUS MINUTIFLORS* SP. NOV. FROM EL PALMITO, DURANGO, MÉXICO (GANESAN 1990). Arrows and bold face type indicate ploidy levels and frequencies with which they were observed.

| *n* = | Number of cells |
|---|---|
| 29 | 3 |
| → **32** | **23** |
| 33 | 10 |
| 34 | 5 |
| 35 | 8 |
| 36 | 8 |
| 37 | 2 |
| 38 | 1 |
| 45 | 3 |
| 46 | 4 |
| 47 | 4 |
| → **48** | **23** |
| 49 | 4 |
| 52 | 1 |
| 60 | 1 |
| 61 | 3 |
| 62 | 4 |
| 63 | 2 |
| → **64** | **20** |
| 65 | 3 |
| 66 | 2 |
| | 134 |

hand pollinated with pollen from the male parent. The flowers were not emasculated for two reasons. First, the presence of some seeds resulting from self-pollinations led to better, more vigorous growth of the ovary thus improving the chances of hybrid seeds developing. Second, when the seeds were harvested, sown and the seedlings grown, seedlings resulting from self-pollinations provided clear examples of the female parent for direct comparison with the putative $F_1$ hybrids, thus facilitating their recognition. A seedling was considered to be a hybrid if it exhibited trait(s) of the male parent not present in the female parent, e.g., anthocyanin leaf markings. For each cross 10, if there were that many, $F_1$ hybrids were grown to maturity. Those that flowered were self-pollinated, and the resulting seeds collected, counted, and the mean seed set calculated (Table 3). An average of 54 capsules was collected and counted for each cross.

## RESULTS AND DISCUSSION

Morphological comparisons revealed that the study populations included 1 population of *M. dentilobus,* 9 of *M. wiensii,* and 4 that

TABLE 3. $F_1$ HYBRID RESULTS FOR TEST CROSSES OF 10 SELECTED NEW COLLECTIONS OF *MIMULUS* FROM WESTERN MEXICO WITH EACH OTHER AND WITH THE 6 REFERENCE POPULATIONS (AND EACH OTHER)—THEIR APPARENT CLOSEST RELATIVES AND GEOGRAPHIC NEIGHBORS. Numerical data are means of seed sets of $F_1$ hybrids. Note: "*h*" indicates non-flowering $F_1$ hybrid; "*f*" indicates combination failed; "—" means combination not tried.

| | | 5324 | 13007 | 12215 | 6209 | 6272 | 6273 | 12206 | 12220 | 12222 | 13008 | 13094 | 13095 | 12197 | 13257 | 12218 | 7169 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference Populations | | | | | | | | | | | | | | | | | |
| *M. dentilobus* | | | | | | | | | | | | | | | | | |
| 5324 | *n* = 16 | 7.8 | 0.6 | *f* | — | 0.0 | — | *f* | — | *f* | — | — | — | 1.9 | *f* | 0.0 | — |
| 13007 | *n* = 16 | 7.6 | 25.5 | *f* | — | *f* | *f* | *f* | *f* | *f* | 0.0 | *f* | *f* | *f* | — | 0.0 | *f* |
| *M. glabratus* v. *fremontii* | | | | | | | | | | | | | | | | | |
| 12215 | *n* = 15 | *f* | *f* | 18.9 | — | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | 0.0 | *f* |
| *M. glabratus* v. *glabratus* | | | | | | | | | | | | | | | | | |
| 6209 | *n* = 31 | — | — | — | 19.9 | — | *f* | — | — | — | *f* | *f* | *f* | — | — | — | *f* |
| *M. wiensii* | | | | | | | | | | | | | | | | | |
| 6272 | *n* = 16 | *f* | *f* | *f* | *f* | 3.9 | 17.8 | 13.0 | 0.6 | 0.0 | 0.0 | 32.0 | *h* | 11.9 | *h* | 0.0 | *f* |
| 6273 | *n* = 16 | — | *f* | *f* | — | 0.0 | 3.8 | — | *h* | — | 0.0 | 0.0 | *h* | *h* | — | *f* | *f* |
| New Collections: | | | | | | | | | | | | | | | | | |
| *M. wiensii* | | | | | | | | | | | | | | | | | |
| 12206 | *n* = 16 | *h* | *f* | *f* | — | 64.8 | — | 26.0 | — | 7.9 | — | — | — | 4.5 | *f* | *f* | — |
| 12220 | *n* = 16 | — | *f* | *f* | — | 0.0 | 5.5 | — | 5.2 | — | 0.0 | 3.2 | 6.9 | 2.9 | *f* | *f* | *f* |
| 12222 | *n* = 16 | *f* | *f* | *f* | — | 72.5 | — | 33.2 | — | 27.8 | — | — | — | 11.8 | *f* | *f* | — |
| 13008 | *n* = 16 | — | *f* | *f* | *f* | 18.1 | 5.6 | *h* | 3.0 | — | 22.5 | 4.5 | 20.2 | 1.1 | *f* | *f* | *f* |
| 13094 | *n* = 16 | — | *f* | *f* | *f* | *h* | *h* | — | *h* | — | 13.7 | 43.6 | 4.6 | 11.9 | *f* | *f* | *f* |
| 13095 | *n* = 16 | — | *f* | *f* | *f* | 28.7 | 13.6 | — | 8.7 | — | 76.5 | 32.6 | 73.2 | 31.6 | *f* | *f* | *f* |
| 12197 | *n* = 16 | *f* | *f* | *f* | — | 7.4 | 5.5 | *f* | *f* | 11.7 | 3.2 | 16.5 | 14.0 | 34.0 | *f* | *f* | *f* |
| *M. yecorensis* | | | | | | | | | | | | | | | | | |
| 13257 | *n* = 32 | *f* | — | *f* | — | *f* | — | *f* | *f* | *f* | *h* | *f* | *f* | *f* | 16.0 | *f* | *f* |
| *M. minutiflorus* | | | | | | | | | | | | | | | | | |
| 12218 | *n* = 32 | *f* | *f* | 0.0 | — | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | *f* | 57.4 | 4.6 |
| 7169 | *n* = 32±, 48±, 64± | — | *f* | *f* | *f* | *f* | *f* | — | *f* | — | *f* | *f* | *f* | 1.3 | *f* | 8.2 | 74.0 |

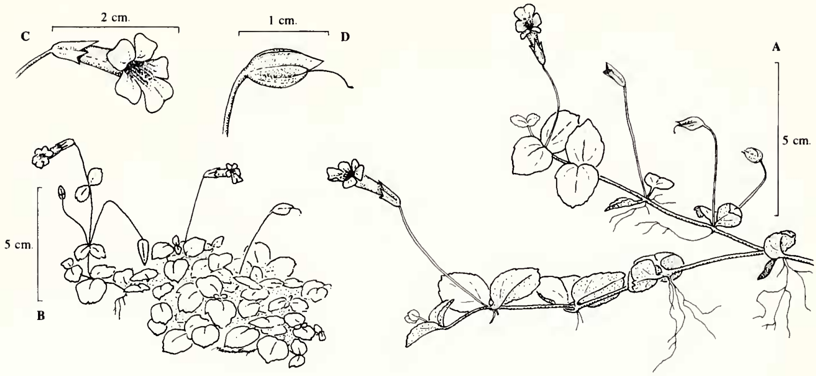


FIG. 1. *Mimulus yecorensis* sp. nov. A. a branch drawn from a live plant grown from seeds of the type collection UT 119,044 B. habit, whole plant. C. flower. D. fruiting calyx.

appeared to be distinctive enough to represent 2 new species (Table 1). One of the latter populations, *13257,* from near Yecora, Mexico, differed markedly from the rest of the complex in its growth habit. The plants formed dense, low-growing mats with delicate flowers rising above the mats on long (2.5–4 cm), slender pedicels (Fig. 1). The other 3 distinctive populations (*7169, 12218,* and *13518*) formed a morphologically similar group that differed from the rest of the complex in having very small flowers (5–6 mm long versus 10–12 mm or more). Also, the plants had erect, delicate, wiry stems (Fig. 2), in contrast to the more succulent, stems of *M. wiensii* and the population from near Yecora.

The cytological studies revealed that all the *M. wiensii* populations were diploid with $n = 16$ chromosomes as was the population of *M. dentilobus* (Table 1). In contrast, the new species were polyploid. The Yecora population had $n = 32$ chromosomes. Of the very small flowered populations two (*12218* and *13518*) had $n = 32$ chromosomes, whereas the third (*7169*) had $n = 32\pm$, $n = 48\pm$, and $n = 64\pm$ chromosomes (Table 2). Population *7169* was so cytologically variable as to suggest much aneuploidy and probably back crossing, as well, among its plants.

The crossing experiments revealed only tenuous relationships among the *M. wiensii, M. dentilobus,* and *M. glabratus* complexes (Table 3). Within the *M. wiensii* complex, the various populations of *M. wiensii* hybridized readily—only 2 failures among the 53 combinations tested (Table 3).

The Yecora population (*13257*) formed only non-flowering $F_1$ hybrids with two of the *M. wiensii* populations. The inability of population *13257* to exchange genes with members of the complex coupled with its striking morphological distinctiveness and tetraploid

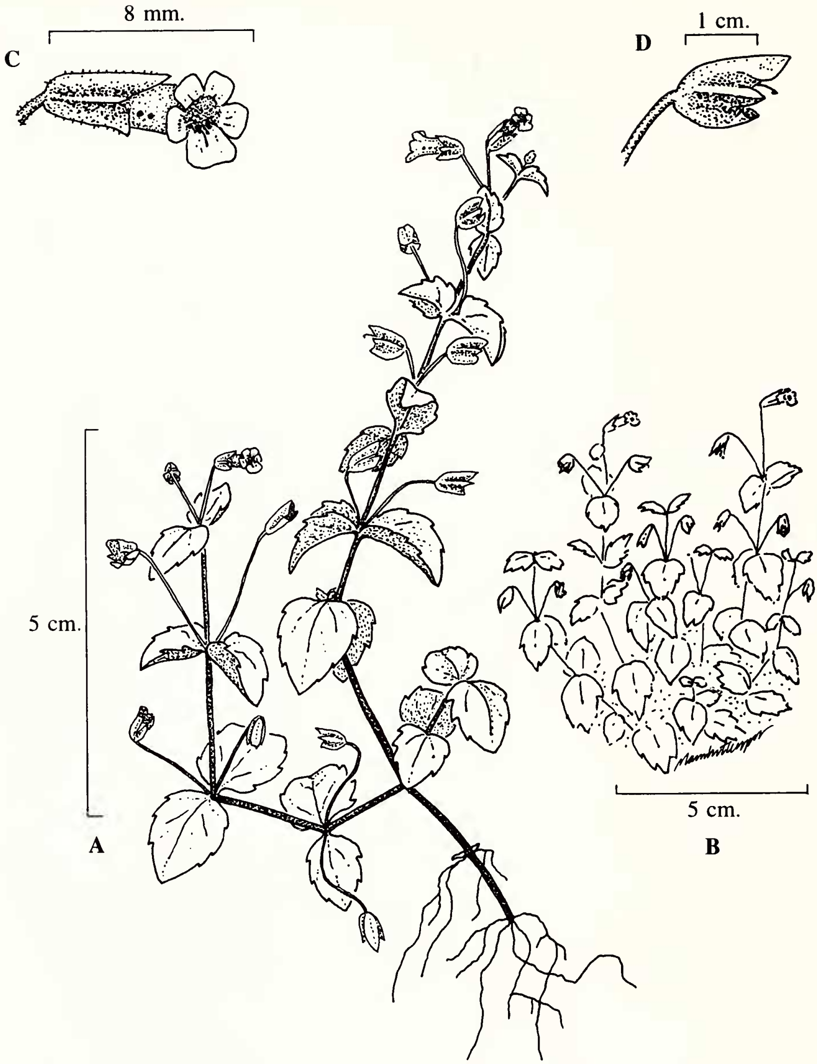


FIG. 2. *Mimulus minutiflorus* sp. nov. A. a branch drawn from a live plant grown from seeds of the type collection UT 119,045. B. habit, whole plant. C. flower. D. fruiting calyx.

chromosome number compared to *M. wiensii* warrants recognizing it as a new species, *M. yecorensis,* named for its region of occurrence.

The very small flowered populations crossed readily with each other (Table 3) but only formed one nearly sterile $F_1$ hybrid with one population of *M. wiensii.* They did not cross with *M. yecorensis,* with which they might have been expected to hybridize on the basis of chromosome numbers (Table 3). Considering its genetic isolation, distinctive morphology, and tetraploid or higher chromosome numbers, I conclude that the very small flowered populations represent a second new species, *M. minutiflorus* named for its distinctive, tiny flowers.

Thus, the *M. wiensii* complex includes in addition to *M. wiensii,* 2 new species, *M. yecorensis* and *M. minutiflorus.*

## The New Species

**Mimulus yecorensis** Vickery, sp. nov. (Fig. 1)—TYPE: MEXICO, Sonora, 17 km east of Yecora on México 16, on mossy banks of small stream, *Dan A. Polhemos s.n.,* 26 April 1982, *Vickery culture 13,257* (holotype: UT; isotypes: UC, DS, US, GH, RSA, MICH, SRSC, MEXU).

Plantae succulentae, humile repentes, tegetes densam formantes; caules virides, prostati ad libere nodos radicantes, ramosissimi usque at 60 cm longos; folia opposita, laeta, ovata ad orbicularia, 1–3 cm longa, 1–3 cm latis, serrulata, palmate 3–5 venis; flores stantes supra tegetes herbarum in gracilibus pedicellis 2.5–4.0 cm longis; calyx quinquelobus, campanulatus in maturite obturbinescens, 5–9 mm longus; corolla bilabiata, 1.3–1.8 cm longa, 1.0–1.3 cm lata, flava, faux punctis ruberis; stylus 1; stigma bilabiatum, sensitivum; stamina 4, didynama, stylo breviores; capsula ovata; semina ellipsoida, 0.3–0.4 mm longa, brunnea fusca; $n = 32$, *Mimulus wiensii* affilis.

Plants low, creeping, forming dense mats. Stems glabrous, green occasionally tinged with red towards apex, up to 60 cm or more in length, and much branched in pairs from nodes. Roots fibrous and slender. Leaves opposite, bright green above and below, ovate to orbicular, 1–3 cm long by 1–3 cm wide, serrulate, and palmately 3–5-veined. Petioles green, glabrous to pubescent along the margins, 1–4 cm long, diminishing in length towards apex. Flowers standing above mat of herbage. Pedicels slender, green occasionally tinged with red, approximately 0.5 mm in diameter, 2.5–4.0 cm long, longer than subtending leaf. Calyx 5-lobed, campanulate becoming obturbinate in maturity, 5–9 mm long, green to tinged with red at base, teeth triangular, upper longest, 2 lower curving inward in maturity. Corolla 5-lobed, 1.3–1.8 cm long, 1.0–1.3 cm wide, bilabiate, yellow, throat with red dots, lobes entire to slightly notched. Style 1, glabrous half again as long as calyx. Capsule ovate, less than half as long as calyx. Seeds ellipsoidal, 0.3–0.4 mm long, dark brown. $n = 32$.

*Distribution.* Known only from the Yecora area, Sonora, Mexico, where it grows on moist stream banks in the pine forest.

**Mimulus minutiflorus** Vickery, sp. nov. (Fig. 2)—TYPE: MEXICO, Durango, km 165.5 on México 40 in ephemerally moist, sunny areas in the pine forest. Elevation 2220 m. *R. K. Vickery Jr. 2901,* 12 May 1976, *culture number 12218.* (holotype: UT; isotypes: UC, DS, US, GH, RSA, MICH, SRSC, MEXU).

Herbae parvae, erectae and glabras; caules filo metalico similes, 5–20 cm alti, cum ramis ad nodos humiliores; folia opposita, ovata, 5–25 mm longa, 5–20 mm lata, serrulata, palmate 3–5 venis; flores in paribis axillaribus; pedicelli puberuli, 1–2 cm longi, virides, saepe rubrotincti; calyx quinquelobus, obturbinatus, 5–6 mm longus in maturite extendens ad 7–8 mm; corolla 5–6 longa, 5–8 mm lata, bilabiata, flava, punctis ruberis in fauce; stylus 1; stigma bilabiatum, sensitivum; stamina 4, didynama, stylo breviores; capsula oblonga, semina ellipsoida, 0.3–0.4 mm longa, fusca brunnea; $n$ = 32, 32±, 48±, 64±; *Mimulus wiensii* affilis.

Plants small, 5–20 cm high, erect, ephemeral annuals. Stems slender, 1–2 mm in diameter, wiry, terete, green occasionally tinged with red, glabrous to puberulent, with opposite branches, rooting at lower nodes. Roots fibrous. Leaves opposite, ovate 5–25 mm long, 5–20 mm wide, serrate, palmately 3–5-veined, green above and below, glabrous at lower nodes to puberulent at upper nodes. Petioles glabrous, green, shorter than the leaves, 5–10 mm long at lower nodes diminishing upwards to 1 mm or less. Flowers in axillary pairs, rarely single. Pedicels puberulent, slender, 0.5 mm or less in diameter, 1–2 cm long, green occasionally tinged with red. Calyx 5-lobed, obturbinate, 5–6 mm long, lengthening to 7–8 mm in maturity, puberulent to nearly glabrous in maturity, green to tinged with red along the ridges, teeth triangular, upper tooth longest, the 2 lower curving inwards in maturity. Corolla 5-lobed, 5–9 mm long, 5–8 mm wide, bilabiate, yellow, throat with red dots, lobes entire. Style 1, glabrous, equaling longest calyx lobe in length. Capsule oblong, less than half length of calyx. Seeds ellipsoidal, 0.3–0.4 mm long, dark brown. $n$ = 32, 32±, 48±, 64±.

*Distribution.* Near the crest of the Sierra Madre in the states of Durango and Sinaloa, Mexico, where it grows in sunny, ephemerally moist little swales.

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